REMARKS

The requisite fee for a three month extension of time and for filing a Request for Continued Examination (RCE) can be charged to Deposit Account No. 02-1818. Any fees that may be due in connection with filing this paper or with this application during its entire pendency may be charged to Deposit Account No. 02-1818. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. 02-1818.

A Change of Correspondence Address accompanies this paper.

A Supplemental Information Disclosure Statement is filed herewith.

References cited in this response are being provided herewith.

An unexecuted DECLARATION of Michael Lindenbaum (herein DECLARATION 8) pursuant to 37 C.F.R. §1.132 accompanies this response. The executed DECLARATION of Dr. Michael Lindenbaum will be provided under separate cover upon receipt.

All Responses and DECLARATIONS of record, including the Response and DECLARATION 1 of Fabijanski filed July 16, 2003; the Response and DECLARATION 2 of Fabijanski, filed April 22, 2004; the Response and DECLARATION 3 of Fabijanski filed January 16, 2005; the Response and DECLARATION 4 of Fabijanski filed November 09, 2005; the Response and DECLARATION 5 of Fabijanksi and DECLARATION 6 of Hadlaczky filed April 30, 2007; and the response and DECLARATION 7 of Fabijanski filed January 31, 2008, responsive to previous Office Actions are incorporated by reference in their entirety herein.

Claims 50-52, 73-79, 81, 84, 87-95, 97-99, 101, 104, 108, 111, 114-115, 117, 119-121 and 128 are pending. Claims 88-91, 94, 98, 99, 120 and 121 are amended to correct minor grammatical errors. Claim 128 is added. Basis for the added claim is found in the original application. No new matter is added.

INTERVIEW WITH EXAMINER

The undersigned thanks Examiner Page and Supervisory Examiner Fox for their courtesy extended in discussing this application and corresponding applications (U.S. Application Serial Nos. 10/287,313 and 11/355,288) in a preliminary telephone interview on June 30, 2008, and also thanks Examiner Page and Examiner Fox for their time and courtesy in further discussing issues in a telephone interview July 1, 2008. The interview was requested to advance prosecution of this and the corresponding applications.

The telephone interviews related to outstanding issues set forth in Office Actions in this and the corresponding applications directed to plant SATACs. It is Applicant's position that 1) the application and corresponding applications describe a "generalizable" and "universal" method of generating SATACs in any eukaryotic species, including plants, and the resulting SATACs; 2) no knowledge of centromeric sequences nor any chromosome sequences is necessary to generate SATACs or in plants; 3) SATACs can be identified based on the presence of structurally identifying characteristics of more heterochromatin then euchromatin interspersed with heterologous DNA; hence identification of the centromere is not necessary to identify a SATAC; 4) the state of the art regarding artificial chromosomes at the time of filing is not apt because such references do not appreciate that which is disclosed in the application, rendering concerns and problems in such art moot; 5) the DECLARATIONS of record evidence generation of SATACs and replication thereof in plants using the methods as described in the instant application; and 6) the application provides all disclosure necessary to generate SATACs, as well as sausage chromosomes, in plants and animals.

Responsive to Applicant's position, Examiner Page reiterated arguments in this Action that, that in order to confirm that a plant SATAC is generated, it is necessary to demonstrate that the SATAC contains a centromere, since a SATAC is an artificial chromosome.

The Applicant disagrees with the Examiner's position that the centromere must be identified by its sequence. No knowledge, nor identification, of the centromeric sequence is required for generation of a SATAC in any species. As discussed in previous responses, the SATAC does contain a centromere duplicated *de novo* from the amplification event inside the cell that produces a dicentric chromosome, and ultimately a SATAC. This event is initiated by introducing DNA into the cell, and looking for cells that contain a SATAC. As described in the application, the integration event occurs upon integration of the DNA into pericentric heterochromatin, either randomly or by targeted integration, and finding the cell in which such event has occurred can be readily achieved by introducing a selectable marker and growing the cells under selective conditions. To identify a SATAC, however, it is **not** necessary to identify the centromere in the SATAC. As described in detail in the specification, in previous responses and further below, a SATAC is an artificial chromosome that is stable and can replicate alongside endogenous chromosomes; it is identified based on

its characteristics as a stable, extragenomic chromosome that is predominately heterochromatin and interspersed with heterologous DNA. By virtue of its replicability, as evidenced by its presence in progency cells, including in transgenic plants and seeds, presence of the centromere can be inferred. It's replicability and stability has been demonstrated in the presence and in the absence of selective agents (as described in the specification and exemplified herein in DECLARATION 8). By virtue of these properties, particularly its stability, the SATAC necessarily has a functioning centromere.

PRELIMINARY REMARKS

It appears from the Examiner's comments in the Office Action that there is confusion regarding the SATACs encompassed by in the claims for generating a transgenic plant. SATACs are a type of artificial chromosome described in the instant application generated *de novo* by methods taught in detail in the application and the priority applications. It respectfully is submitted, however, that the instant application describes two different methods for generating artificial chromosomes. Many of the Examiner's comments relate to the second method of in vitro assembly of artificial chromosomes, which is a method that is distinct and separate from the method of generating SATACs as taught in the instant application.

The first method, the "Preparation of SATACs" is described in detail in the application (see *e.g.* page 33, line 19 to page 36, line 23). Examples and Figures (*e.g.* Figures 2 and 3), which is the subject of claims in the instant application, and claims in corresponding applications and priority applications. As discussed in detail in previous responses, this method involves introducing nucleic acid into cells, by targeted or non-targeted integration into the pericentric heterochromatin, which initiates amplification events leading to the generation of a *de novo* centromere, growing the cells and looking for the amplified structures, including SATACs, precursors thereof, and other amplified structures, such as sausage chromosomes, and then further manipulating cells or SATACs and/or isolating them. The method requires **NO** knowledge of any chromosomal sequence. Further, the method is generally applicable to any eukaryotic species, including plants. In particular, the specification describes that an advantage of the method herein, as exemplified by generation of mammalian SATACs, is that the construction of mammalian artificial chromosomes was "hindered by the lack of an isolated, functional, mammalian centromere and uncertainty regarding the requisites for its production and stable replication" (see *e.g.*, at page 4, lines 9-

11). Hence, the method provided in the instant application overcomes this problem by providing a method that permits construction of species-specific artificial chromosomes *de novo* (see *e.g.* at page 5, lines 3-5), without requiring any knowledge of any sequences of any component parts of an artificial chromosomes of any species. As stated in previous responses, one adds DNA to cell, particularly DNA with a selectable marker, grows the cells under selective conditions, and "poof" these events occur resulting in SATACs and sausage chromosomes.

The second method, which is not the subject of the instant claims, is a method involving the *in vitro* construction of artificial chromosomes. This method **does not** generate SATACs. Rather, the method relies on "assembling the structural and functional elements that contribute to a complete chromosome capable of stable replication and segregation alongside endogenous chromosomes in cells" (at page 42, lines 5-8). To do so, the *in vitro* method of chromosome assembly can make use of the SATACs generated in the first method above to identify and isolate the component parts for the *in vitro* assembly method (see *e.g.*, at page 43, lines 5-9). For practice of this method, some knowledge of chromosomal sequences and structures is required. (It is noted that no knowledge of the centromeric sequences is required; the centromeres can be derived from the SATACs). This method, however, is **not** the subject of any pending claims in this or any copending application.

It is emphasized that the instant claims are directed to SATACs, generated *de novo*, and not to artificial chromosomes assembled *in vitro*. Hence, any comments by the Examiner that refer to any portion of the specification directed to this latter *in vitro* method are irrelevant to SATACs and to methods of making SATACs, used in the instantly claimed method of generating transgenic plants. For example, the Examiner at page 12 of the Office Actions rebuts Applicant's comment that the sequence of origins of replication are not required to practice the method by stating that "[t]his is not persuasive because the origin of replication is a required element of the SATAC as defined by Applicant on page 17.... It is noted that Applicants provide exemplary sequences for the origin of replication of mammalian cells." It respectfully is submitted that Page 17 of the specification referred to by the Examiner provides the definition of an "*in vitro* synthesized artificial chromosome." Similar comments are made by the Examiner at page 9 of the Office Action. For example, the Examiner states that "Applicant appeared to fully recognize the necessity of providing the evidence for the presence of the centromere for mammalian SATACs as the specification

details the identification with centromere specific probes, and also related the importance of cloning centromeric sequences for the construction of artificial chromosomes (see page 88 3rd paragraph and pages 44 -45)." This is not correct. The method of generating SATACs DOES NOT involve cloning centromeric sequences. The description on pages 44-45 details construction of *in vitro* assembled chromosomes. Further, as discussed herein below, there is no requirement to identify a SATAC using any centromere-specific antibody or probe.

As discussed in detail in previous responses, no knowledge of any chromosomal sequence is required to practice the method of generating SATACs in any cells, including mammals or plants. None were required in the specification as of its filing date to generate SATACs from mouse cells, nor SATACs from any eukaryotic species.

DECLARATION

A Declaration of Michael Lindenbaum (DECLARATION 8) is provided herewith. Michael Lindenbaum is not an inventor of the application. He holds a Ph.D., and , is representative of a person of skill in this art with respect to performing experiments in accord with a disclosed protocol. He is employed as Vice-President of Technology Development of Agrisoma Biosciences. Chromos Molecular Systems, Inc., an original assignee of record, is an owner of Agrisoma, to whom the subject matter of this application is licensed.

The Declaration is provided to evidence practice of the method and identification of SATACs under non-selective conditions. The Declaration describes generation of SATACs in soybean using the methods as taught in the specification, including all steps as taught in the application. The Declaration describes introduction of DNA containing hygromycin as a selectable marker into soybean calli by bombardment and growth of cells under selective condition. The Declaration describes identification of cells that had undergone amplification events by southern blot analysis. The Declaration further demonstrates stability of SATACs by growth of plants under non-selective condition and identification of SATACs in T1 seeds. The results depict identification of SATACs by Fluorescence in situ hybridization using probes to co-localize amplified heterochromatin and heterologous DNA. The Declaration also evidences the resulting chromosomal structures as autonomous chromosomes. Hence, the Declaration shows that, just as taught in the specification, SATACs generated in soybean are characterized and can be identified by their existence as stable, extragenomic chromosomal structures having amplified heterochromatin interspersed with heterologous DNA.

The results provided in the Declaration were generated following introduction of a DNA containing rDNA from Arabadopsis, but the Declaration states that similar results were obtained using an rDNA from soybean. Hence, the Declaration shows that the source of rDNA used in the method is not plant species specific. Accordingly, the Declaration shows that by practicing the method exactly as described in the specification, amplification of pericentric heterochromatin and generation of chromosomal structures containing amplified pericentric heterochromatin, including SATACs, occurs in soybean.

I. REJECTION OF CLAIMS 50-52, 73-79, 81, 84, 87-95, 97-99, 101, 104, 108, 111, 114-115, 117 AND 119-121 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 50-52, 73-79, 81, 84, 87-95, 97-99, 101, 104, 108, 111, 114-115, 117 and 119-121 are rejected under 35 U.S.C. §112, first paragraph, because it is alleged that the specification does not provide guidance for any plant artificial chromosome, sequences, or methods of making the same, or any plant cell comprising any artificial chromosomes.

Applicant respectfully traverses this rejection for reasons of record, and as further discussed below. The rejections are substantially the same as in previous Office Actions, which have been addressed in detail in corresponding responses, each incorporated by reference herein. The main points set forth in the Examiner's rejection, and Applicant's rebuttal thereto, are addressed below. The case law has been discussed in previous responses and is incorporated by reference herein.

Rebuttal

1) The Examiner urges that the method described in the DECLARATIONS of record evidencing generation of plant SATACs is different from and not supported by the specification. The Examiner urges that the methods of identification of the SATACs described in the DECLARATIONS are not disclosed in the specification. The Examiner further urges that the DECLARATIONS do not evidence that the SATACs contain a functioning centromere, since the centromere was not identified by its sequence, nor was it evidenced to be stable under non-selective conditions.

Applicant respectfully disagrees for reasons of record and as further discussed herein below.

For example, the Examiner states on page 19 of the Office Action :

> The specification outlines the use of BrdU as well as a series of steps involved in creating megachromosomes and gigachoromosomes, sorting cells containing each and treating the cells to induce the breakage of dicentric chromosomes. Applicants continue to assert that the exact same methods are

used, but also continue to show differences in even basic steps of the method. As stated above, mouse chromosome 7 was identified using G-banding patterns, and mouse centromeric regions were identified using alphoid satellite DNA probes. Neither tool was available for similar identification of particular chromosomes or centromeric regions in plants. So the methodology of identification and tracking the SATACs is considerably different, and one of skill in the art would not have been guided by the specification to modify as Applicants have done. More than routine experimentation is required and more steps are required to be sure one has a functioning centromere and thus, actually have a functioning embodiment of the invention.

The Examiner further states on page 8 – 9 of the Office Action that:

> One major difference between the detailed specification as it regards mammalian SATAC generation and the declarations are the methods used to identify the various chromosomes and chromosome structures. The application and specification do not rely on primary constrictions to identify centromeres, but instead, disclose and use known centromeric sequences for the identification of sausage chromosomes as well as other SATACs. Furthermore, the specification uses banding patterns specific to particular chromosomes to identify which chromosome is incorporated into the SATACs. The specification does not outline any different method for chromosome identification for plants, does not address the necessary differences in plants, and does not disclose the structures and features that would be necessary for such identification. The declarations rely on the use of 18s rDNA probes and the probe for the selective marker to identify the same chromosomal structures. The problem with this method is that neither of these probes identify or would allow one of skill in the art to recognize that any part of the plants endogenous centromere has been incorporated into the structure as shown in great detail in the specification for mammalian chromosomes...

First, it respectfully is submitted that description of the SATAC characterization provided in the specification was exquisitely detailed precisely to set forth the identifying characteristics of SATACs. Hence, the specification sets forth the use of multiple different methods used to identify SATACs, including G-banding, C-banding, immunofluorescence, electron microscopy, *in situ* hybridization, including fluorescence in suit hybridization (FISH), and Southern Blot. The specification also provides exhaustive detail of all of the intermediate structures that are produced by the method and the process by which chromosomes are produced from formerly dicentric chromosomes. The specification describes that BrdU **and/or** a selective agent can be used to destabilize the chromosomes to result in formation of the SATAC (see *e.g.*, at page 33, lines 8-15). *There is no requirement for BrdU destabilization to generate a SATAC.* Further, the specification uses antibody-specific probes against the centromere to confirm that the SATAC contains a centromere. In addition, experiments evidencing stability also were provided to establish that the SATACs

contained a functional centromere (see *e.g.* page 17, lines 12-14 and at p.93, lines 9-12). Based on the results of these studies, described in detail in the Examples and in the Figures (*e.g.* Figure 2), the specification describes that the identifying characteristics of a SATAC is that it is an extragenomic, stable chromosomal structure that contains amplified heterochromatin interspersed with the heterologous DNA (at page 16, lines 22-24; at page 17, lines 1-4; at page 18, lines 24-26; and at page 19, lines 4-8). Once the identifying characteristics are known and described, it is not necessary for one of skill in the art to repeat the detailed characterization of SATACs, including the analysis and identification of all intermediate chromosome structures. It is enough to identify a SATAC using any of the exemplified ways taught in the specification that permits identification of SATACs based on the identifying characteristics provided in the specification.

As described and exemplified in the specification, one way to identify a SATAC is by *in situ* hybridization, including fluorescence *in situ* hybridization, for chromosomal structures co-stained for amplified heterochromatin and the heterologous DNA. The specification describes that sequences contained in the pericentric heterochromatin, which by practice of the method are amplified, include rDNA and/or satellite DNA (see *e.g.* at page 29, lines 11-28). The Examples use mouse satellite DNA probes to identify amplified heterochromatin, however, the Examples also indicate the presence of rDNA in these regions (see *e.g.*, at page 97, lines 16-24). In particular, the Examples describe identification of SATACs by *in situ* hybridization for amplified heterochromatin. For example, Example 6 describes that *in situ* hybridization using mouse major satellite DNA as a probe is sufficient to identify SATACs by permitting identification of the amplified heterochromatin (see *e.g.* at page 94, lines 10-16) . Despite the fact that Example 6 also describes that the hybridization signal co-localized to the signal produced using the human anti-centromere serum, the probe to the mouse satellite DNA was not used to identify the centromere; it was used to identify the amplified heterochromatin, which is an identifying feature of SATACs. An antibody to the centromere also can be used, but this is not necessary, since evidence of a centromere can be evidenced by function as a stable chromosome as discussed below. Further, the Example shows that *in situ* hybridization for the amplified heterochromatin and the heterologous DNA co-localized (see *e.g.* at page 95, lines 5-7). The DECLARATIONS of record, including DECLARATION 8 provided herein, identifies SATACs exactly as described in the specification, *i.e.* using *in situ* hybridization for co-localization of amplified heterochromatin

and heterologous DNA. As taught in the specification, The DECLARATIONS of record use probes to rDNA to identify the amplified heterochromatin. There is no requirement that pericentric heterochromatin must be identified using satellite DNA probes.

Despite the fact that the Examiner acknowledges at page 19 of the response that *in situ* hybridization had been performed successfully on some plants at the time of filing, the Examiner comments that the full scope of the claims is directed to all plant cells and all plant tissues. First, Applicant respectfully submits that *in situ* hybridization is just one way set forth in the application that SATACs can be identified. The fact that the DECLARATIONS do not evidence all ways is not dispositive that SATACs were not identified and produced exactly as described. The exact method used to identify the SATAC is not a recited element of the claims. Second, *in situ* hybridization was an available technique in plants, and using such methods, Applicant has identified SATACs in three diverse plant species, Brassica and Nicotina (DECLARATIONS 5 and 7) and soybean (DECLARATION 8). Demonstration of the identification of SATACs in three diverse plant species using methods as taught in the specification evidences the applicability of the teachings of the specification within the full scope of the claims.

The Examiner further rebuts that "visualization of a single gene marker was not routine in the art using fluorescence *in situ* hybridization at the time of filing in plants." Applicant respectfully disagrees. As discussed in the previous response and in DECLARATION 7 of Fabijanski, fluorescence *in situ* (FISH) techniques were available at the time of filing the earliest priority application (see *e.g.*, Leitch *et al.* (1991) *Genome*, 34:329-333; Fukui *et al.* (1994) *Theor. Appl. Genet.*, 87:893-899; Jiang *et al.* (1995) *Proc. Natl. Acad. Sci.* USA, 92:4487-4491; Murata and Motoyoshi (1995) *Chromosoma*, 104:39-43; Matsuyama *et al.* (1996) *Genome*, 39:941-945; Zhong *et al.* (1996) *Chr. Res.*, 4:24-28; and Schubert I and Wobus U (1985) *Chromosoma*, 92:143-148). Also, the Examiner is reminded that MPEP 2144.03 states:

> The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well- known" in the art. *In re Ahlert*, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . .

The facts of which the Examiner is taking notice are conclusory and are not capable of instant and unquestionable demonstration as being "well-known" in the art. MPEP 2144.03 continues:

> If justified, the examiner should not be obliged to spend time to produce documentary proof. If the knowledge is of such notorious character that official notice can be taken, it is sufficient so to state. *In re Malcolm*, 129 F.2d

> 529, 54 USPQ 235 (CCPA 1942). If the applicant traverses such an assertion the examiner should cite a reference in support of his or her position.

In this instance, if the rejection is maintained that *in situ* hybridization, including FISH, was not routine at the time of filing, documentation supporting this position, respectfully is requested.

The specification also describes the use of Southern Blot to identify the copy number of the integrated DNA sequence to evidence its amplification in the method. The existence of multiple copies of the integrated DNA is indicative of the amplification methods that result by practice of the method. Accordingly, cells that have undergone the amplification process versus those that have merely integrated the DNA into an area of the chromosome can be distinguished by analyzing cells by Southern hybridization for those that contain multiple copies of the integrated exogenous DNA (see *e.g.*, at page 19, lines 16-19; at page 84, lines 3-6; at page 86, line 16-18; at page 96, lines 26-29). This is further exemplified in the DECLARATIONS of record. For example, DECLARATION 5 demonstrates by Southern blot that the selectable marker is present at greater than 20 copies, evidencing that large-scale amplification of the exogenous DNA had occurred. Similar results are depicted in DECLARATION 8 provided herewith.

Further, the specification describes that to confirm that such a structure is a SATAC, it is sufficient to identify it based on its characteristics as an extragenomic, stable structure. This is described in the specification (see *e.g.* at page 16, lines 22-29; at page 17, lines 12-14 and at p.93, lines 9-12). Thus, it is not necessary, nor required, to have knowledge of the centromeric sequence in order to identify that a SATAC has a functioning centromere as stated by the Examiner at page 12 of the Office Action. The use of anti-centromere antibodies used in the specification were used in the detailed characterization of SATACs as described above. Once it was determined the identifying characteristics of SATACs, which is universal and shared across species, it is not necessary to identify a SATAC based on knowledge of centromere sequence. **There is no teaching in the specification that knowledge of centromeric sequences are required to identify SATACs.** Further, as to the Examiner's comments on page 22 of the Office Action, there is absolutely no requirement of isolate and sequence centromeric sequences from plants and use those sequences as probes in identifying the SATAC. This was not even taught or described in the specification with respect to identification of mammalian SATACs. It appears that the Examiner is relying on the teachings in the specification with respect to in vitro assembly of artificial chromosomes.

As discussed above, the teachings in the specification of methods of generation of SATACs and their identifying characteristics is a different method from the in vitro assembly method taught at page 42, line 4 to page 48, line 10.

In fact, the Examiner recognized that SATACs containing a functioning centromere can be identified based on their stability. For example, at page 9-10 of the Office Action by the Examiner states:

> ...Furthermore, the specification provides definitive evidence of an artificial chromosome with a fully functioning centromere on page 82 of the specification where it is fully recognized that long term culturing in non-selective conditions shows that the chromosome is stably maintained....

The Examiner, however, urges that the DECLARATIONS of record evidencing stability are not persuasive because:

> ...DECLARATION 7 discloses repeated methods of DECLARATION 5 along with new photos showing Mendelian inheritance of the SATACs. Fabijanski discloses two generations both of which are constantly exposed to the negative selection agent which would necessarily lead to the maintenance of the fragment for survival of the plant cell. It is not necessary for the fragment to contain a fully functional centromere or indeed a centromere at all for such maintenance, as plasmid and other vectors have often been maintained in the cell under the exposure of a negative selection agent. Evidence is still lacking for the stable maintenance of a fully functioning centromere. Regardless, no such evidence and no such teachings exist in the instant specification. It is repeated that the instant specification does disclose the incorporation of a fully functional centromere in mammalian cells, and does so on page 82 of the instant specification with generations under no selection pressure.

Based on this reasoning, the Examiner further states at page 16-17 of the Office Action that DECLARATION 2 of Fabijanski demonstrating that SATACs in calli were stably maintained in culture for over six months is not persuasive. The Examiner reasons that:

> ...the growth conditions included a negative selection medium. As discussed above, the presence of a DNA fragment containing a resistance gene maintained in plants or cells with the application of a negative selection agent does not give evidence of a functioning centromere.

Applicant respectfully submits that the specification at page 17, lines 12-14 teaches that stability can be assessed when "at least about 85%, preferably 90%, more preferably 95% of the cells retain the chromosome. Stability is measured in the presence of a selective agent. Preferably these chromosomes are also maintained in the absence of a selective agent." Thus, there is no requirement that stability be assessed under non-selective agent. Further, the

Examiner's comments that stability of a SATAC, and evidence of a functioning centromere, can only be assessed in the absence of negative selection is not scientifically sound. During mitosis, the centromere is essential for chromosome segregation between daughter cells. Chromosomes that do not contain a functional centromere will not be included in either of the daughter cells. The presence of a selective agent does not make the centromere function. Rather, the purpose of the selective agent is simply to enrich for cells containing SATACs. Even in the presence of a selective agent, if the centromere is not functional, then the chromosome will not be maintained during mitosis and will be lost.

Thus, even though the DECLARATIONS of record evidence maintenance of SATACs in the presence of a selective agent, maintenance was achieved over **thousands of mitotic divisions**. Upon division of cells, if the SATAC did not contain a functioning centromere it would have been lost over this many cell divisions. This was not the case. Further, the DECLARATIONS also showed stability of the SATACs through meiosis by its transmission to a second generation plant. It therefore is respectfully submitted that the Examiner's statement that DECLARATION 5 and 7 of Fabijanski only "evidences two generations" is not reflective of the results shown because both mitotic and meiotic stability was evidenced. The DECLARATION 7 of Fabijanski, in referring to DECLARATION 5, clearly states that "selection and analysis of the tobacco protoplasts were performed 14 to 21 days after transfection, meaning thousands of mitotic divisions occurred and the SATAC was still maintained." In addition, the DECLARATION evidenced meiotic transmission into a second generation plant. Thus, as stated by Fabijanski in DECLARATION 7 the fact that transmission of SATACs in mitosis and meiosis is normal evidences its stability. Again, if this rejection is maintained that Applicant has not shown that the SATAC contains a functioning centromere by virtue of its stability and maintenance across generations, documentation supporting this position, respectfully is requested.

Notwithstanding the above, Applicant provides herewith DECLARATION 8 of Lindenbaum, discussed in detail above, which evidences **stability of SATACs under non-selective conditions**. The SATACs were generated using the methods as described in the application by introducing heterologous DNA into soybean and growing the cells under hygromycin selection and recovering plants and identifying those with a SATAC. The plants were then grown in the absence of selection and seed were analyzed for the presence of a SATAC. Thus, the DECLARATION evidences that the SATAC is stably maintained

mitotically and meiotically in the absence of selection and found in the second generation of a plant derived from a plant cell containing a SATAC. The specification describes identification of SATAC by it shared structural features as containing amplified heterochromatin interspersed with heterologous DNA and as being a separate autonomous chromosome. Accordingly, the DECLARATION 8 further depicts that the SATAC generated by the methods described in the above-captioned application contains a functional centromere by virtue of its maintenance across generations under no selection pressure.

At page 17 of the Office Action, the Examiner rebuts Applicant's argument that there is no better test of chromosome stability than its presence in generation after generation. While the Examiner was persuaded that chromosome stability evidences a functional centromere in mammalian cells (*e.g.* page 9-10 of the Office Action cited above), the Examiner was not persuaded that Applicant had evidenced such stability in plants. Specifically, the Examiner stated:

> Applicants have not shown stable transmission in subsequent generations beyond the second generation, have not shown such generations in the absence of negative selection pressure, and have not shown such stable transmission in a high enough number of plants to suggest the presence of a fully functioning centromere in the SATACs.

Applicant respectfully submits that Applicant has done all of these things. 1) Stable transmission of SATACs has been demonstrated across thousands of mitotic cells divisions, into seeds of plants, and into a fully regenerated plant. 2) Further, the fact that SATACs are identified in cells after weeks to months of culture and mitotic division, even in the presence of a selective agent, means that the SATAC contains a functional centromere. In addition, the growth of plants in the absence of selection and analysis of seeds for the presence of a SATAC unequivocally establishes that the SATAC contains a functioning centromere. 3) Finally, the generation of SATACs in plants using the methods as taught in the specification was demonstrated in three diverse species of plants (*e.g.* Nicotina, Brassica and soybean).

In particular, DECLARATIONS 5, 7 and 8 demonstrate generation of SATACs based on the teachings of the specification and using steps exactly as taught in the specification. For example, the DECLARATIONS demonstrate introduction of heterologous DNA containing a selectable marker into plant cells (*e.g.* L-PPT or hygromycin) as taught, for example, at page 6, lines 15-17 and at page 33, lines 23-25. It was introduced into cells in the presence of a sequence targeted to the pericentric heterochomatin (*i.e.* rDNA; see *e.g.* at page 29, line 11-28). Cells were grown under selective conditions (see *e.g.*, page 34, lines 3-

4). In particular, the specification provides extensive details of the types of plant cells that can be introduced with DNA and the methods used to introduce DNA into plant cells (see *e.g.*, beginning at page 54 under the heading "**Introduction of heterologous DNA into plants**"). For example, DECLARATIONS 5 and 7 demonstrate introduction of heterologous DNA into plants by PEG-mediated transfer and DECLARATION 8 demonstrates introduction of DNA by microprojectile bombardment (*e.g.* biolistic) as taught in the specification. Finally, as discussed in detail above, the DECLARATIONS demonstrate methods of identifying the amplification events that result by practice of the methods and identification of SATACs characterized by amplified heterochromatin interspersed with the heterologous DNA using southern blot and *in situ* hybridization methods. Further, the DECLARATIONS demonstrate confirmation of stable SATACs by virtue of their maintenance and transmission through mitosis and meiosis. Thus, the DECLARATIONS of record demonstrate practice of the method of generating SATACs in plants as taught in the specification.

2) The Examiner continues to rely on the teachings of various cited references to evidence that it would be unpredictable to generate SATACs in plants.

Applicant does not disagree with the Examiner that the references describe the state of the art of artificial chromosomes. In fact, as discussed above, the specification acknowledges problems in reconstructing artificial chromosomes, even with respect to mammalian artificial chromosomes (see *e.g.* at page 4, lines 6-11). The method described in the application, however, overcomes these problems because it is based on a pioneering invention based on the discovery of and exploitation of a fundamental process common to all chromosomes. As taught in the application, it was discovered that chromosomes undergo amplification processes that, when exploited as described in the instant application, can be employed to produce what applicant has called satellite artificial chromosomes (SATACs). The application generically identifies the intrinsic large-scale amplification that occurs. The application teaches how to exploit this to generate satellite artificial chromosomes in **any cell** by introducing nucleic acid into cells, which nucleic acid either randomly integrates into the pericentric heterochromatin or is targeted to such region, growing the cells and looking for the amplified structures, which constitute precursors to the SATACs or SATACs, and then further manipulating cells or SATACs and/or isolating them. The application specifically teaches (*i.e.*, page 9) that the process can be applied to any species, including plants and

animals, to produce SATACs. Dr. Gyula Hadlaczky, has been awarded the prestigious Széchenyi Award in 2000 in his native country, Hungary, and more recently the Denis Gabor Award in 2007, for the work that is the subject of the above-captioned application. This evidences the pioneering nature of the claimed subject matter.

The references do not discuss this discovery, including the method of generating artificial chromosome structures *de novo*, nor the resulting SATACs. Hence, as stated in previous responses, **the references do not partake in the benefit of the teachings of the instant application**. Rather, the references are specific to discussion of individual components of artificial chromosomes, and thus their applicability relates to the predictability of generation of *in vitro* assembled artificial chromosomes. As discussed above, the method of generating SATACs does not involve *in vitro* assembly, but rather involves a process of *de novo* generation. Accordingly, as discussed in the previous, no knowledge of any chromosomal sequences or function, including centromere sequences or other sequences, are required to practice the method. The method taught in the instant application for generation of SATACs overcomes the problems described in these references by discovery of a fundamental process common to all eukaryotic results that permits *de novo* generation of artificial chromosomes that are stable, extragenomic chromosomal structures and that permit carrying and expression of gene sequence for the generation of transgenic animals and plants.

Further, as discussed in the previous response, DECLARATIONS of record, including DECLARATION 8 provided herewith, unequivocally establish that the method as taught in the instant application works in plants. The DECLARATIONS use methods as described in the specification to generate SATACs in plants, and to identify the resulting SATACs. The DECLARATIONS demonstrate that, by following the teachings of the specification, the methods operate as claimed for the generation of plant SATACs. The DECLARATIONS show that the instant application teaches method that are universally applicable to the generation of SATACs in eukaryotes, including the generation of plant SATACs. Further, DECLARATION 7 demonstrates the reproducibility of the method, since several research groups, by following the teachings of the specification, have generated SATACs from plants.

3) The Examiner cites the specification at pages 126-128 to allege that the specification gives no guidance as to what changes would be made with the use of plant cells to generate SATACs using the methods as taught in the specification.

In particular, the Examiner states at page 6 of the Office Action:

> ...The specification goes into great detail in pages 126-128 regarding the differences of particular cell lines and how that would affect the methods used. However, the specification gives no guidance as to what changes would be made with the use of plant cells. While discussing the differences of chromosomes number and chromosome size, there is no discussion of content and no discussion of the very different chromosome size and DNA content of plant cells. These differences are furthermore not discussed or addressed in the declarations of record. The assertion by Applicants that no knowledge of these plant specific structures and sequences is required is in contrast to the detailed teachings that enable Applicants for the generation of mammalian SATACs. (similar comments also are made at Page 15 of the Office Action)

It appears from the Examiner's comments that the Examiner believes that knowledge of chromosome number and chromosome size is essential for the practice of the method. This is not correct. It respectfully is submitted that pages 126-128 is a part of Example 10 that exemplifies a particular way to **isolate** SATACs from cells based on their atypical based content and/or size. Isolation of SATACs is not required to generate SATAC, nor to identify cells containing SATACs. Further, it is possible to introduce SATACs into cells without ever isolating SATACs, for example, by cell fusion (see *e.g.*, claims 52, 74, 77, 81, 101, 108 and 111). Further, the description at pages 126-128 relating to particular cells lines and culturing procedures refers to the choice of cell in order to permit *large-scale production* of artificial chromosomes. The specification renders it clear that any cell can be used to for *de novo* formation of SATACs using the method as described in the specification (see *e.g.* at page 51, lines 28 to page 52, line 10; at page 128, lines 7-10). It is a universal method that is applicable to any cell and any eukaryotic species. Some cells, however, may be better suited for large scale production based on considerations involving isolation. The instant claims are directed to methods of generating a transgenic plant and do not require large-scale isolation of SATACs. Hence, the teachings of the specification at pages 126-128 are irrelevant to the instant claims.

Further, the method of isolating SATACs described in Example 10 is not the only way to isolate SATACs. Exemplary methods of isolating SATACs are described in detail in the specification beginning at page 41 under the heading "Isolation of Artificial Chromosomes." Notwithstanding this, the specification describes that SATACs can be isolated by fluorescence-activated cell sorting (FACs) by taking advantage of the nucleotide base content of the SATACs, by virtue of their high heterochromatic DNA content, which differs from any other chromosome in a cell (see *e.g.* at page 41, lines 7-11). Isolation of

chromosomes in plants by FACs was well known to one of skill in the art at the time of filing the instant application and priority applications (see *e.g.*, de Laat and Blaas (1984) Theor Appl. Genet., 67:463-467). Furthermore, it was well known at the time of filing the instant application that heterochromatin, including plant heterochromatin, has a distinct base content (Guerra (2000) Genetics and Molecular Biology, 23:1029-1041 and references cited therein; Martin et al. (1988) Heredity, 61:459-467; and Leemann et al. (1983) Experimental Cell Research, 147:419-429).

4) *The Examiner continues to urge that DECLARATION 6 of Hadlaczky is an opinion.*

Specifically, the Examiner states:

> Applicants urge that DECLARATION 6 of Hadlaczky was provided to evidence the universality of the chromosomal processes involved in the generation of SATACs and that the method is based on the fundamental process of chromosomal replication common to plants and animals (pages 19-20 of response).
>
> This is not persuasive as it pertains to enablement because the detailed methods outlined by the specification as they apply to mammalian cells including human, mouse and hamster is not in question. It is recognized in the art that there are many similarities in the sequences and proteins involved in these processes in mammalian cells. It is likewise recognized as cited in previous office actions that the sequences and proteins involved in these processes in plants are in fact, different. Therefore, it is merely an opinion stated by Hadlaczky that these same methods should work in plants as well as mammalian systems. Hadlaczky does not take these differences into account, and it is not sufficient to state the cellular processes are fundamentally the same when the elements required for the invention are known to be different.

Applicant respectfully disagrees for reasons of record and as further discussed herein. As discussed in the previous response, Dr. Hadlaczky, an inventor of this application, attested to the universality of the method of SATAC generation, and the fact that it is a fundamental process that is common to eukaryotes, including animals and plants. As noted above, Dr. Gyula Hadlaczky, has been awarded the prestigious Széchenyi Award in 2000 in his native country, Hungary, and more recently the Denis Gabor Award in 2007, for the work that is the subject of the above-captioned application. The method is not dependent on knowledge of sequences of chromosomal structures.

The specification teaches that it is a universal method and is applicable to generation of *de novo* species-specific artificial chromosomes. An entire section of the specification is directed to "hosts" for which heterologous DNA can be introduced in order to generate

SATACs, in particular plant hosts. Methods of DNA introduction in plants are taught. Hence, the realization of the universality of the method was evident at the time of filing the instant application. In fact, the pioneering nature of these satellite artificial chromosomes has been acknowledged and recognized by the US Patent Office, which granted three patents to family members of this application. These include patents with claims to SATACs per se, human SATACs, cells that contain SATACs per se, methods of producing a product in a cell containing a SATAC without reference to species of satellite artificial chromosome or cell. Issued claims cover SATACs from any species and include claims to cells that contain SATACs. Neither SATACs nor cells are limited to particular species. Issues of scope were fully vetted during prosecution of the issued patents.

II. The Rejection of Claims 50-52, 73-79, 81, 84, 97-99, 101, 104, 108, 111, 114, 115, 117 and 119-121 Under 35 U.S.C. §112, First Paragraph – Written Description

Claims 50-52, 73-79, 81, 84, 87-95, 97-99, 101, 104, 108, 111, 114-115, 117 and 119-121 are rejected under 35 U.S.C. §112, first paragraph for alleged lack of written description, because it is alleged that the specification does not describe the subject matter in such a way as to convey to one skilled in the relevant art that the inventor(s) had possession of the claimed subject matter at the time the application was filed. The claims are rejected for reasons of record that the specification allegedly only provides guidance for a satellite artificial chromosome in mammalian cells, specifically mouse cells, and does not provide guidance for any plant artificial chromosomes, sequences, or methods of making same, or any plant cells comprising any artificial chromosomes.

Applicant respectfully traverses this rejection for reasons of record, and as further discussed below. The rejections are substantially the same as in previous Office Actions, which have been addressed in detail in corresponding responses, each incorporated by reference herein. The main points set forth in the Examiner's rejection, and Applicant's rebuttal thereto, are addressed below.

Relevant Law

The case law has been discussed in previous responses and is incorporated by reference herein. The purpose of the written description requirement is to ensure that the patent applicant had possession of the claimed subject matter at the time of filing of the application In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an

express or an implicit disclosure. To satisfy the written description requirement, "the applicant does not have to utilize any particular form of disclosure to describe the subject matter claimed, but the description must clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *In re Alton*, 76 F.3d 1168, 1172 (Fed. Cir. 1996). The applicant by the disclosure must "convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention," *Vas-Cath Inc.*, 935 F.2d at 1563-64.

For example, the Federal Circuit, in adopting the Guidelines for Examination of Patent Applications under 35 U.S.C. §112, first paragraph stated that the written description requirement can be met by:

> ...show[ing] that an invention is complete by disclosure of sufficiently detailed, **relevant identifying characteristics . . . *i.e.*, complete or partial structure**, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics [emphasis added] *Enzo Biochem Inc. v. Gen-Probe Inc*, 323 F.3d 956 (Fed. Cir. 2002).

Further, in *Falker-Gunter Falkner et al. v. Inglis et al.* 448 F.3d 1357 (Fed. Cir. 2006), the Federal Circuit held that (1) examples are not necessary to support the adequacy of a written description; (2) the written description standard may be met (as it is here) even where actual reduction to practice of an invention is absent; and (3) there is no per se rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure.

Analysis

As discussed in previous responses, Applicant has more then adequately evidenced possession of satellite artificial chromosomes, including plant SATACs, based on the description set forth in the specification as of its earliest filing date. As discussed previously, the specification describes in detail the *de novo* method of generating SATACs by introducing heterologous DNA into cells, growing the cells under selective conditions and identifying cells that have integrated the heterologous DNA resulting in amplification events and production of a SATAC. The specification describes that the method is applicable to all species, including plants, (see *e.g.* at page 9, lines 14-18) and results in *de novo* production of species-specific artificial chromosomes (see *e.g.* at page 5, lines 3-5 and at page 51, line 27 to page 52, line 10). The description in the specification renders it clear that Applicant's were in possession of a method of generating SATACs in plants, and the resulting SATACs,

because there is an entire section in the specification devoted to exemplary plant hosts and methods of introducing heterologous DNA into plants (see*.e.g.* at page 51, line 27 to page 52, line 10 and at page 54, line 1 to page 55, line 3 under the section headed "Introduction of heterologous DNA into plants.")

The specification clearly describes the relevant identifying characteristics of SATACs, which is shared across all species of SATACs by virtue of practice of the method and the resultant amplification events and *de novo* formed centromere that result in production of a SATAC. For example, the application provides a detailed description of the common features of satellite artificial chromosomes that applies whether the satellite artificial chromosome is derived from an animal, plant or other cell. The application makes clear that the common attributes possessed by the members of the genus of satellite artificial chromosomes are relatively invariant: they have more heterochromatin than euchromatin and generally contain duplicated segments of DNA, which includes highly repetitive DNA, such as, for example, pericentric heterochromatic DNA or satellite DNA (see *e.g.*, page 7, lines 15-20). Furthermore, all arise by the amplification events as described in the application. The application describes that, except for the heterologous nucleic acid such as a selectable marker or other foreign DNA, the SATACs contains only non-protein-encoding heterochromoatin (see page 7, lines 15-20). The application describes that this megachromosome (*i.e.* SATAC) is stable and can replicate and segregate alongside an endogenous chromosome (see, *e.g.*, page 16, lines 22-35; page 34, line 23).

Based on these identifying characteristics, the specification describes how to identify a satellite artificial chromosome, for example, by C-banding and/or fluorescence *in situ* hybridization (FISH) using labeled probes to visualize the amplified heterochromatin and the heterologous DNA. The application provides detailed descriptions of the structure of satellite artificial chromosomes, including the schematics in the figures (see *e.g.* Figure 2).

Rebuttal to Examiner's Comments

1) *The Examiner urges that the detailed description of SATACs and generating SATACs as well as the deposited materials applies to mammalian cells and the generation of SATACs in mammalian cells. The Examiner further rebuts Applicant's argument of record that the methods are applicable to plants.*

The Examiner states:

> This is not persuasive because a mere assertion does not adequately describe nor show possession of the claimed embodiment. While several

generic techniques are listed in the specification, no mention is made of potential modifications that would be required for any plant species.

This is not correct. Applicant did much more then merely assert that the specification describes generation of SATACs in plants; the specification **does** describe the generation of SATACs in plants. The description in the specification is generic to generation of SATACs in any eukaryotic species. As stated in previous responses, the specification clearly describes that the method of generating SATACs is the same and can be performed in any cell to generate species-specific SATACs (see *e.g.* at page 5, lines 3-5; at page 9, lines 14-18; and at page 51, line 27 to page 52, line 10). The method steps are the exact same. For example, the specification states at page 9, lines 14-18 that methods of producing SATACs are provided and that "[t]hese methods are applicable to the production of artificial chromosomes containing centromeres derived from any higher eukaryotic cell, including mammals, birds, fowl, fish, insects and plants." Further, the specification describes in detail exemplary plant hosts and methods of introducing DNA into plants in accordance with practice of the method.

Further, the specification describes relevant identifying characteristics of SATACs, *i.e.* that it is an extragenomic, stable chromosomal structure that contains amplified heterochromatin interspersed with the heterologous DNA (at page 16, lines 22-24; at page 17, lines 1-4; at page 18, lines 24-26; and at page 19, lines 4-8). These relevant identifying characteristics are common to the genus of SATACs, including animal and plant SATACs. The specification describes methods that can be used to identify SATACs based on these characteristics (*e.g. in situ* hybridization, southern blot, assessment of transmission and stability). As noted above, the U.S. Patent Office considered that the genus of SATACs was sufficiently described since claims were granted to SATACs per se.

It respectfully is submitted that there is no requirement that all species within a genus be reduced to practice, nor that the specification include examples of all species within a genus. In this instance, Applicant exemplified the generation of SATACs in mammalian cells and characterized the resulting SATACs and described the identifying characteristics of SATACs. This description is generic to all SATACs. Exemplary SATACs with these identifying characteristics were deposited as cell lines. It is not dispositive that Applicant did not deposit cell lines containing SATACs of all claimed species. This is not a requirement for satisfaction of the written description requirement.

In addition, no modification of the method steps are required to practice the methods as claimed. This is exemplified by the DECLARATIONS of record and DECLARATION 8

provided herewith that evidence generation of SATACs in plants using the methods as taught in the specification, and identifying the SATACs based on methods taught in the specification. There were no modification of any of these steps in the DECLARATIONS. Hence, the DECLARATIONS evidence that the methods described in the specification, as described, are applicable to generation of SATACs in plants and that the resulting SATACs have the same identifying characteristics as described in the specification.

2) *The Examiner states that there is no disclosure of any plant sequences for a SATAC, and therefore there is no description of the required structures of the instant application. The Examiner further urges that Applicant's arguments that the structural features common to the genus are not persuasive.*

The Examiner states at page 25 and 26 of the Office Action that:

> ...the specification does not describe plant centromeres or plant origins of replication at all, much less "in detail". Applicant has been repeatedly invited to point out where in the specification such description is located. There is no description of sequences and there is no description of what part of the centromere is required to function.
>
> ...the specification does not describe the structural features of the centromeres or the origins of replication that are required for function. Indeed these structural features were unknown at the time of filing, particularly as it regards the plant sequences and structural features. As Applicants have identified centromeres and origins of replication as required structures for SATAC function, it is incumbent on Applicants to adequately describe the structural features for the claimed embodiments, or at the very least identify the structural features that each species shares that is required for function. While these structural features have been described and disclosed for mammalian centromeres and origins of replication in the form of SEQ IDs, these structures have not been disclosed for plant centromeres or origins of replication, nor have any identifying characteristics of such sequences been identified or described by Applicant.

It respectfully is submitted that Applicant has stated on the record that, in accordance with the method described in the specification, no knowledge of any chromosomal sequences, including the sequence of the centromere, is required to practice the method described in the specification to generate SATACs and identify SATACs. As discussed above, the method of generating SATACs described in the specification is a *de novo* method. It is different from the in vitro assembly method also described in the specification where knowledge of the centromere, origin of replication and other chromosomal components are required to practice the method. Hence, the Examiner's statement that Applicant provided sequences of mammalian centromeres and origins of replication in the form of SEQ IDs for the generation

of mammalian SATACs is incorrect. **Further, Applicant requests that the Examiner point out where in the specification SEQ ID NOS for mammalian centromeres and origins of replication were provided in the specification that must be known for the generation of mammalian SATACs.**

The method of generating SATACs is advantageous **because** no knowledge of sequence nor knowledge of function of any chromosomal components is required. By introduction of DNA into cells and introduction into the pericentric heterochromatin, an amplification event occurs resulting in the *de novo* duplication of an endogenous centromere and the production of SATACs and intermediates and precursors of SATACs. The specification describes the problems with conventional methods of generating artificial chromosomes, even in mammalian cells (see *e.g.*, at page 4, lines 6-11), and provides an alternative *de novo* method where knowledge of chromosomal sequences is not required. The specification describes relevant identifying characteristics of the resulting SATACs that permits identification of such SATACs. The Examples identify SATACs based on these characteristics such as by using *in situ* hybridization for co-localization of amplified heterochromatin and heterologous DNA. Similar methods are described in the DECLARATIONS of record and DECLARATION 8 provided herewith for identification of SATACs generated in plants. Further, the specification **does not** describe that it is necessary to identify a centromere by its sequence in order **to identify a SATAC**. The specification renders it clear that SATACs are extragenomic, stable structures, and can be identified based on their stability. The use of anti-centromere antibodies, such as used in one instance in the Examples, is only one way to show that the SATAC contains a functioning centromere. Assessment of stability and maintenance of the SATAC under selective and non-selective conditions is another way.

Accordingly, since knowledge of chromosomal sequences are not required for practice of the method, nor identification of SATACs, in any species, the Examiner's reliance on Amgen v. Chugai Pharmaceuticals is inapt for reasons of record set forth in the last response. In this instance, relevant identifying characteristics of SATACs are provided and evidence possession of SATACs, including plant SATACs.

3*) The Examiner urges that Applicant's description of the method as described in the specification is not persuasive.*

Specifically, it is stated at pages 26-27 of the Office Action that:

> Applicants urge that the DECLARATIONS demonstrate that "all of one of skill in the art has to do is introduce a DNA fragment into a cell, grow the cell under selective conditions and 'poof' SATACs and/or precursors or intermediates thereof are produced by the cell (page 28 of response)
>
> This is not persuasive because the specification and DECLARATIONS do not teach introducing just any fragment of DNA into a cell, and in fact have strict requirements on targeting sequences as well as the requirement of selectable marker to be included in the introduced DNA fragment.

It respectfully is submitted that the method of generating a SATAC by the *de novo* method described in the specification, in any species, is exactly as described by Applicant and does involve simply introducing a DNA fragment into a cell, growing the cell under selection conditions and identifying those cells that contain a SATAC or precursors or intermediates such as a sausage chromosome. As discussed in previous responses, the specification describes that the DNA contains a selectable marker to permit growth of the cells under selective conditions and identification of the resulting SATACs containing the amplified introduced heterologous DNA. The choice of selectable marker can be any selectable marker that permits selection of cells. The specification also describes that the DNA can be non-targeted, but that the use of DNA that is targeted to the pericentric heterochromatin increases the chance of amplification events. For example, the specification at page 29, lines 11-17 states that SATACs are generated by integration of the heterologous DNA into the pericentric heterochromatin. The specification describes that "the frequency of incorporation can be increased by targeting to these regions, such as by including DNA, including but not limited to, rDNA or satellite DNA, in the heterologous fragment that encodes the selectable marker." There is no requirement to use a targeting sequence.

Notwithstanding the above, it is unclear the relevance of the Examiner's comment. The specification clearly describes the steps of the method and that the heterologous DNA contains a selectable marker that can, if desired, be targeted to the pericentric heterochromatin by including a targeting sequences. Example 6 evidences practice of the method as described by introduction of a heterologous DNA encoding hygromycin and a λCM8 DNA that targets to the pericentric heterochromatin. Similarly, just as described in the specification, the DECLARATIONS of record evidence generation of SATACs in plants using a heterologous DNA containing a selectable marker (L-PPT or hygromycin) and a sequence that targets to the pericentric heterochromatin (rDNA). Thus, the methods set forth in the DECLARATIONS of record are in accord with the description provided in the

specification, regardless of whether one considers such description to have strict requirements (which it does not).

4) *The Examiner urges that Applicant's argument that the physical embodiments of a plant SATAC described in the specification and demonstrated in the DECLARATIONS are not persuasive because these embodiments are not contained within the original specification and are not thus identified in the original specification. Therefore, the Examiner urges that the DECLARATIONS do not reveal the "entire plant artificial chromosome hybrid."*

First, it is unclear what the Examiner means by "entire plant artificial chromosome hybrid." The DECLARATIONS of record were provided to evidence generation of SATACs in plants and precursors and intermediates thereof. As discussed in detail above and in previous responses, the specification describes that SATACs, including mammalian and plant SATACs and SATACs generated in other eukaryotes, share a common structural feature achieved by virtue of practice of the *de novo* method that results in the amplification processes. By virtue of the *de novo* amplification method and duplication of the endogenous centromere, SATACs contain a species-specific centromere. SATACs are stable, extragenomic chromosomes that are predominately heterochromatic and are interspersed with heterologous DNA. These relevant identifying characteristics, their description in the specification, and exemplification in mammalian SATACs and in SATACs generated in plants as demonstrated in DECLARATIONS have been discussed in detail throughout this response and previous responses.

5) *The Examiner urges that satisfaction of the written description is not satisfied for the generation of plant SATACs because satisfaction of the written description requirement cannot be achieved by providing description of relevant structural features shared by all SATACs. The Examiner continues to urge that knowledge of centromere sequences is required to identify SATACs, and that the description of mammalian centromeric sequences as probes to show the existence of centromeres in the generated mammalian SATACs is not representative of plant centromeres.*

Applicant respectfully disagrees. As discussed above in review of the relevant law, written description can be satisfied by describing the relevant identifying characteristics shared by all members of the genus. Applicant described by detailing description and Figures the relevant identifying characteristics of SATACs. The relevant identifying characteristics and shared structural features were exemplified in mammalian SATACs, but are common to

the genus of SATACs. It is not required, nor necessary, to identify SATACs from any species using any probes that are specific to the centromere.

6) The Examiner states that it is unclear which methods are required to practice the invention with plants, as with mammalian cells because an application of BrdU was employed for destabilization. The Examiner urges that no mention of BrdU stabilization is made in any of the DECLARATIONS of record and it is not clear from the specification whether or not this step is required.

Applicant respectfully submits that the specification renders it clear that BrdU stabilization is not required to practice the method in any eukaryotic species, including mammals or plants. For example, the specification states at page 9, lines 4-13 that various methods can be used to generate cell lines that contain chromosomes that carry stable heterochromatic chromosomes (*i.e.* SATACs). Such methods include cell fusion, growth on selective medium and/or BrdU treatment or other treatment with other genome destabilizing agents. The DECLARATIONS of record, including DECLARATION 8 provided herewith evidence that, in accord with the description in the specification, SATACs and precursors and intermediates of SATACs were generated by growth of cells under selective conditions.

III. Rejection for Obviousness-Type Double Patenting 92, 95, 99, 114-115

Claims 92, 95, 99 and 114-155 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting over claims in various co-owned applications. The Office Actions state that the rejections include rejection of claims 114-155. It is assumed that this is a typographical error and that the rejection includes rejection of claims 114-115. If this is not correct, Applicant respectfully requests clarification. The relevant law is discussed in previous responses and is incorporated by reference herein.

Rejection of Claims 92, 95, 99 and 114-115 over Application No. 10/287,313

Claims 92, 95, 99 and 114-115 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting over claims 52 and 66 of copending Application No. 10/287,313. It respectfully is submitted that claims 52 and 66 have been cancelled from copending Application No. 10/287,313. Hence, rejection of claims 92, 95, 99 and 114-115 is moot.

Rejection of Claims 92, 95, 99 and 114-115 over Application No. 11/284,877

Claims 92, 95, 99 and 114-115 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting over claim 63 of copending Application No.

11/284,877. It respectfully is submitted that claim 63 has been cancelled from copending Application No. 11/284,877. Hence, rejection of claims 92, 95, 99 and 114-115 is moot.

* * *

In view of the election, amendments and remarks herein, examination on the merits respectfully is requested.

Respectfully submitted,

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